

**Claims:**

1. An oligonucleotide molecule for use in the detection of mRNA transcribed from the E6 gene of a human papillomavirus, the oligonucleotide comprising  
5 any one of sequence numbers 1-133.

2. An oligonucleotide primer for use in the detection of mRNA transcribed from the E6 gene of a human papillomavirus, the oligonucleotide primer being  
10 selected from:

(i) a NASBA P1 primer comprising one of sequence numbers 2, 4, 8, 11, 14, 17, 20, 22, 25, 28, 31, 34, 37, 39, 42, 45, 48, 50, 52, 57, 60, 63, 65, 69, 71,  
15 75, 78, 81, 84, 87, 90, 92, 96, 98, 100, 105, 107, 111, 113, 115, 121, 126, 127, 128 or 129;

(ii) a NASBA P2 primer comprising one of sequence numbers 1, 3, 7, 10, 13, 16, 19, 21, 24, 27, 30, 33, 36, 38, 41, 44, 47, 49, 51, 56, 59, 62, 64, 68, 70,  
20 74, 77, 80, 83, 86, 89, 91, 95, 97, 99, 104, 106, 110, 112, 114, 120, 103, 131, 132 or 133;

(iii) a PCR primer comprising one of sequence numbers 1, 3, 7, 10, 13, 16, 19, 21, 24, 27, 30, 33, 36, 38, 41, 44, 47, 49, 51, 56, 59, 62, 64, 68, 70, 74, 77, 80, 83, 86, 89, 91, 95, 97, 99, 104, 106, 110, 112, 114, 120, 2, 4, 8, 11, 14, 17, 20, 22, 25, 28, 31, 34, 37, 39, 42, 45, 48, 50, 52, 57, 60, 63, 65, 69, 71,  
25 75, 78, 81, 84, 87, 90, 92, 96, 98, 100, 105, 107, 111, 113, 115, 121, 126, 127, 128, 129, 130, 131, 132 or 133.  
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3. An oligonucleotide primer according to claim  
35 2 which is a NASBA P1 primer having the sequence AATTCTAATACGACTCACTATAGGGAGAAGG-SEQ, wherein SEQ represents any one of sequence numbers 2, 4, 8, 11,

- 46 -

14, 17, 20, 22, 25, 28, 31, 34, 37, 39, 42, 45, 48,  
50, 52, 57, 60, 63, 65, 69, 71, 75, 78, 81, 84, 87, 90,  
92, 96, 98, 100, 105, 107, 111, 113, 115, 121, 126,  
127, 128 or 129.

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4. An oligonucleotide primer according to claim  
2 which is a NASBA P2 primer having the sequence  
GATGCAAGGTCGCATATGAG-SEQ wherein SEQ represents any  
one of sequence numbers 1, 3, 7, 10, 13, 16, 19, 21,  
10 24, 27, 30, 33, 36, 38, 41, 44, 47, 49, 51, 56, 59,  
62, 64, 68, 70, 74, 77, 80, 83, 86, 89, 91, 95, 97,  
99, 104, 106, 110, 112, 114, 120, 130, 131, 132 or  
133.

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5. An oligonucleotide probe for use in the  
detection of mRNA transcribed from the E6 gene of a  
human papillomavirus comprising one of sequence  
numbers: 5, 6, 9, 12, 15, 18, 23, 26, 29, 32, 35, 40,  
43, 46, 53, 54, 55, 58, 61, 66, 67, 72, 73, 76, 82,  
20 85, 88, 93, 94, 101, 102, 103, 108, 109, 116, 117,  
118, 119, 122, 130, 131, 132 or 133.

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6. An oligonucleotide primer-pair for use in  
the detection of mRNA transcripts from the E6 gene of  
HPV 16, comprising one of the following combinations:

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an oligonucleotide primer comprising sequence number 1  
and an oligonucleotide primer comprising sequence  
number 2;

an oligonucleotide primer comprising sequence number 3  
and an oligonucleotide primer comprising sequence  
number 4;

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an oligonucleotide primer comprising sequence number 7  
and an oligonucleotide primer comprising sequence  
number 8;

- 47 -

an oligonucleotide primer comprising sequence number 10 and an oligonucleotide primer comprising sequence number 11;

5 an oligonucleotide primer comprising one of sequence numbers 126, 127, 128 or 129 and an oligonucleotide primer comprising sequence number 1 or sequence number 3; or

10 an oligonucleotide primer comprising sequence number 2 or sequence number 4 and an oligonucleotide primer comprising one of sequence numbers 130, 131, 132 or 133.

7. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 18, comprising one of the following combinations:

15 an oligonucleotide primer comprising sequence number 13 and an oligonucleotide primer comprising sequence number 14;

20 an oligonucleotide primer comprising sequence number 16 and an oligonucleotide primer comprising sequence number 17;

25 an oligonucleotide primer comprising sequence number 19 and an oligonucleotide primer comprising sequence number 20; or

an oligonucleotide primer comprising sequence number 21 and an oligonucleotide primer comprising sequence number 22.

30 8. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 31, comprising one of the following combinations:

35 an oligonucleotide primer comprising sequence number 24 and an oligonucleotide primer comprising sequence number 25;

- an oligonucleotide primer comprising sequence number 27 and an oligonucleotide primer comprising sequence number 28;
- 5 an oligonucleotide primer comprising sequence number 30 and an oligonucleotide primer comprising sequence number 31;
- an oligonucleotide primer comprising sequence number 33 and an oligonucleotide primer comprising sequence number 34; or
- 10 an oligonucleotide primer comprising sequence number 36 and an oligonucleotide primer comprising sequence number 37.

9. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 33, comprising one of the following combinations:
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- an oligonucleotide primer comprising sequence number 38 and an oligonucleotide primer comprising sequence number 39;
- 20 an oligonucleotide primer comprising sequence number 41 and an oligonucleotide primer comprising sequence number 42; or
- an oligonucleotide primer comprising sequence number 44 and an oligonucleotide primer comprising sequence number 45.
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10. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 35, comprising one of the following combinations:
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- an oligonucleotide primer comprising sequence number 47 and an oligonucleotide primer comprising sequence number 48;
- 35 an oligonucleotide primer comprising sequence number 49 and an oligonucleotide primer comprising sequence number 50; or

an oligonucleotide primer comprising sequence number 51 and an oligonucleotide primer comprising sequence number 52.

- 5           11. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 52, comprising one of the following combinations:

10           an oligonucleotide primer comprising sequence number 56 and an oligonucleotide primer comprising sequence number 57; or

an oligonucleotide primer comprising sequence number 59 and an oligonucleotide primer comprising sequence number 60.

- 15           12. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 58, comprising one of the following combinations:

20           an oligonucleotide primer comprising sequence number 62 and an oligonucleotide primer comprising sequence number 63;

an oligonucleotide primer comprising sequence number 64 and an oligonucleotide primer comprising sequence number 65.

- 25           13. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 51, comprising one of the following combinations:

30           an oligonucleotide primer comprising sequence number 104 and an oligonucleotide primer comprising sequence number 105; or

an oligonucleotide primer comprising sequence number 106 and an oligonucleotide primer comprising sequence number 107.

14. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 56, comprising one of the following combinations:

- 5 an oligonucleotide primer comprising sequence number 110 and an oligonucleotide primer comprising sequence number 111;  
an oligonucleotide primer comprising sequence number 112 and an oligonucleotide primer comprising sequence  
10 number 113;  
an oligonucleotide primer comprising sequence number 114 and an oligonucleotide primer comprising sequence number 115;  
an oligonucleotide primer comprising sequence number  
15 120 and an oligonucleotide primer comprising sequence number 121.

15. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 39, comprising one of the following combinations:

- 20 an oligonucleotide primer comprising sequence number 80 and an oligonucleotide primer comprising sequence number 81;  
25 an oligonucleotide primer comprising sequence number 83 and an oligonucleotide primer comprising sequence number 84; or  
an oligonucleotide primer comprising sequence number 86 and an oligonucleotide primer comprising sequence  
30 number 87.

16. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of HPV 45, comprising one of the following combinations:

an oligonucleotide primer comprising sequence number 89 and an oligonucleotide primer comprising sequence number 90;

5 an oligonucleotide primer comprising sequence number 91 and an oligonucleotide primer comprising sequence number 92;

an oligonucleotide primer comprising sequence number 95 and an oligonucleotide primer comprising sequence number 96;

10 an oligonucleotide primer comprising sequence number 97 and an oligonucleotide primer comprising sequence number 98; or

an oligonucleotide primer comprising sequence number 99 and an oligonucleotide primer comprising sequence number 100.

15 17. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of group B HPV, comprising one of the following combinations:

an oligonucleotide primer comprising sequence number 68 and an oligonucleotide primer comprising sequence number 69;

25 an oligonucleotide primer comprising sequence number 70 and an oligonucleotide primer comprising sequence number 71; or

an oligonucleotide primer comprising sequence number 74 and an oligonucleotide primer comprising sequence number 75.

18. An oligonucleotide primer-pair for use in the detection of mRNA transcripts from the E6 gene of group C HPV, comprising the following combination:

an oligonucleotide primer comprising sequence number 77 and an oligonucleotide primer comprising sequence number 78.

5           19. An oligonucleotide primer-pair according to any one of claims 6 to 18 which comprises a NASBA P1 primer and a NASBA P2 primer.

10           20. A primer-pair according to claim 19 wherein the NASBA P1 primer includes the sequence AATTCTAATACGACTCACTATAGGGAGAAGG at the 5' end.

15           21. A primer/probe set comprising a primer-pair according to any one of claims 6 to 20 and at least one oligonucleotide probe specific for amplification products generated using the primer-pair.

20           22. A method of detecting HPV mRNA in a test sample suspected of containing HPV which comprises performing an amplification reaction on a preparation of nucleic acid isolated from the test sample to amplify a portion of the mRNA transcribed from the E6 gene of HPV, wherein the amplification reaction is performed using a primer-pair according to any one of  
25           claims 6 to 18.

30           23. A method according to claim 22 which comprises performing RT-PCR to amplify a portion of the mRNA transcribed from the E6 gene of HPV.

24. A method according to claim 126 which comprises performing NASBA to amplify a portion of the mRNA transcribed from the E6 gene of HPV .

35           25. A method according to claim 24 which comprises:



(a) assembling a reaction mixture comprising a primer set as defined in any one of claims 6 to 18, an RNA directed DNA polymerase, a ribonuclease that hydrolyses the RNA strand of an RNA-DNA hybrid without hydrolysing single or double stranded RNA or DNA, an RNA polymerase that recognises said promoter, and ribonucleoside and deoxyribonucleoside triphosphates;

(b) incubating said reaction mixture with a preparation of nucleic acid isolated from a test sample suspected of containing HPV under reaction conditions which permit a NASBA amplification reaction; and

(c) detecting and/or quantitatively measuring any HPV-specific product of the NASBA amplification reaction.

26. A method according to claim 25 wherein step (c) comprises real-time detection of an HPV-specific product of the NASBA amplification reaction.

27. A method according to claim 25 or claim 26 wherein the reaction mixture further comprises a molecular beacons probe oligonucleotide and the formation of any HPV-specific NASBA product in the NASBA reaction is monitored by detecting fluorescence from the fluorescent moiety included in the molecular beacons probe.

28. A method according to claim 25 or claim 26 which comprises the further step of capturing the NASBA reaction product by hybridisation to a probe oligonucleotide attached to a solid support.

29. A reagent kit for use in the detection of HPV by NASBA, the kit comprising an oligonucleotide primer-pair as defined in claim 19 and optionally an enzyme mixture comprising an RNA directed DNA

polymerase, a ribonuclease that hydrolyses the RNA strand of an RNA-DNA hybrid without hydrolysing single or double stranded RNA or DNA, and an RNA polymerase that recognises the promoter sequence present in at least one NASBA P1 primer oligonucleotide included in the reagent kit.